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Review

An overview of microplastics in oysters: Analysis, hazards, and depuration

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ABSTRACT

Microplastic pollution has become an emergent global environmental issue because of its ubiquitous nature and everlasting ecological impacts. In marine ecosystems, microplastics can serve as carriers to absorb various contaminants and the ingestion of microplastics in oysters is of concern because they can induce several adverse effects. The analytical process of microplastics in oysters commonly consists of separation, quantification, and identification. Quantification of microplastics is difficult since information regarding the analytical methods is incoherent, therefore, standard microplastic analytical methods for shellfish should be established in the future. The depuration process can be used to reduce the level of microplastics in oysters to ensure safe consumption of oysters and longer depuration time facilitates improved depuration efficacy. In summary, this review aims to help better understand microplastic pollution in oysters and provide useful suggestions and guidance for future research.

1. Introduction

Plastics are a suite of synthetic organic polymers derived from the polymerization of monomers extracted from hydrocarbons. Owing to their excellent physicochemical properties, such as wear and corrosion resistance, light weight, durability, convenience, and low price, plastics are widely used in industry and daily life (Chen, Li, & Wang, 2021; Xu, Ma, Ji, Pan, & Miao, 2020). Worldwide plastic production, which is exponentially growing, reached 370 million tons in 2019, with a forecast of 33 billion tons by 2050 (Teng et al., 2021b; Xu et al., 2020). Despite this high plastic production, only about 14% of plastic waste is recycled. The remainder is either incinerated with energy recovery or released into the environment in the manner of landfilling and littering (Systemiq, 2022). With the increase in plastic production, plastic waste derived from plastic products has increased dramatically, causing plastic pollution to attract worldwide attention and become one of the most severe environmental issues.

Owing to their non-biodegradability, larger plastic items break down into smaller plastic fragments, namely microplastics (MPs), during long-term degradation. Generally, MPs are defined as synthetic polymer particles < 5 mm in size (Thompson et al., 2009) and can be categorized into primary and secondary MPs according to their sources and production ways. Primary MPs are manufactured as minute plastic pellets used in cosmetics and personal care products, such as facial scrubs and toothpaste, whereas secondary MPs are mainly derived from conventional larger plastics, such as plastic containers, nets, and tires (Li et al., 2018b,c) that decompose into small MPs under abrasion and degradation.

Oysters are sessile, filter-feeding species that feed by continuously straining particulate matter from seawater through their gills. MPs can be ingested accidentally or intentionally by oysters because of their small dimensions and close resemblance to natural food (Lozano-Hernández et al., 2021). According to Wootton et al. (2022), MPs are found in 94.4% of oysters globally, with an average of 1.41 ± 0.33 per gram of soft tissue (wet weight). Numerous studies have reported deleterious effects of plastic accumulation on oysters, such as oxidative stress (Teng et al., 2021a), metabolic dysfunction (Teng et al., 2021b), and reproductive toxicity (Gardon et al., 2018). It should also be noted that MPs can enter the food chain through trophic transfer and bioaccumulation, and ultimately reach individuals, thereby affecting food safety and human health (Fig. 1). MPs have the potential to translocate to human tissues and trigger localized immune responses. Animal studies and in vitro assays in human cell lines have demonstrated that MPs can pose threats, such as genotoxicity and cytotoxicity in human peripheral blood lymphocytes (Çobanoğlu et al., 2021); and, oxidative stress, reproductive endocrine disruption, and toxicity in

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gastrointestinal, liver, and neuronal systems (Expósito et al., 2022). Therefore, it is necessary to quantify the bioaccumulation of MPs in oysters and estimate the potential adverse effects of MPs on oysters and humans.

Oysters simultaneously play an important economic role in coastal regions, where they serve as popular and abundant commercial seafood products that are consumed in large quantities by people. At the international level, oysters are largely consumed as ready-to-eat food with guaranteed freshness and high quality (Felici et al., 2020); this is a result of avoiding the negative effects of processing or cooking on the quality and taste of oysters. However, owing to their extensive filter-feeding activities, slow-moving characteristics, and wide distribution, oysters tend to have a higher incidence of contaminant enrichment. Depuration of contaminants in oysters is important to ensure their safe consumption. The process of depuration involves placing oysters in seawater disinfected using ozone, ultraviolet light, etc., to eliminate harmful substances (Chen et al., 2022a; Chen et al., 2022b). In the European Union, depuration is mandatory to reduce the risk of food poisoning caused by foodborne pathogenic bacteria (Anon, 2004). Therefore, depuration can potentially be used to reduce the levels of MPs in oysters before human consumption.

In this review, available published studies investigating the accumulation of MPs in oysters are systematically retrieved and collected to summarize well-established analytical methods for the digestion, quantification, and identification of MPs. Subsequently, we review and discuss the direct and indirect hazards of microplastic exposure in detail. Special attention is paid to the depuration of MPs accumulated in oysters. Finally, the prospects and challenges of microplastic studies are discussed. This review aims to not only provide a comprehensive review of microplastic pollution in oysters, but also provide useful suggestions and guidance for future research.

2. Methods

A systematic review of the literature on MPs was conducted using ScienceDirect, Wiley Online Library, SpringerLink, and Web of Science databases. The title, abstract, and keywords were searched using the following terms: “Microplastics” and “nanoplastics” or “plastics” or “contamination” or “oysters” or “bivalves” or “shellfish” or “extraction” or “quantification” or “toxicity” or “hazard” or “impacts” or “adsorption” or “contaminants” or “depuration” or “purification” or “egestion”. The retrieval and analysis of most literature focused on scientific articles written in English that were published after 2020. Some articles prior to 2020 were also cited in this review due to their relevance and importance in this field. In addition, from the literature, many articles were focused on the analysis of MPs, including MPs separation, quantification, and identification. Because there is large variability in the methodologies used when detecting MPs in oysters, to improve the accuracy of the data, research concerning MPs analysis was summarized and compared.

3. Analysis of MPs in oysters

The analysis of MPs in oysters is important since the content of MPs in oysters can laterally reflect the level of MPs contamination in the marine environment. The analytical methods of MPs include extraction, quantification, and identification. Extraction is the key to accurate quantification of MPs content in oysters, however, different extraction methods contribute to variance in results. Therefore, there is a need in exploring a method that ensures the highest extraction efficiency. In the following parts, various analytical methods will be summarized and compared to optimize the analytical process of MPs.

3.1. Extraction of MPs

Before quantification, the separation of MPs from samples should be considered. Separating MPs ingested by oysters requires an efficient extraction method that removes all organic material and soft tissues, and isolates the particles from the sample matrix without damaging the polymer integrity. Therefore, in the following sections, various digestion methods used to extract MPs from oysters are compared to determine the optimal digestion process (Table 1).

3.1.1. Acid digestion

Acid digestion using a range of digestive reagents, such as HNO_3 and HCl, is typically used to isolate MPs from oysters. HNO_3 is capable of molecular cleavage and rapid decomposition of organic materials; and is conventionally used to digest soft tissues and organic compounds present in bivalves to extract MPs. Aung et al. (2022) demonstrated that HNO_3 could completely digest the organic tissues of oysters, and Severini et al. (2019) reported that digestion with HNO_3 yielded higher digestion efficacies, with > 98% removal of organic materials. HCl is relatively gentler and less detrimental to MP isolation than HNO_3.

However, acid digestion is the least suitable method because some of

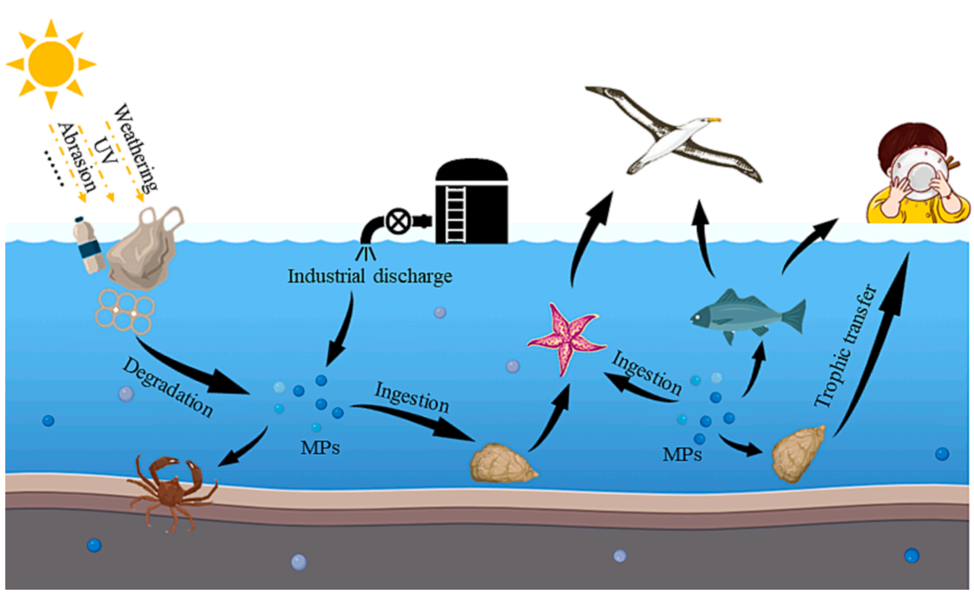

Fig. 1. Sources and transport of microplastics in marine environment.

Table 1
Size, shape, type, abundance, and analysis of microplastics in oysters.

Location	Species	Analysis			Microplastics				Reference
		Digestion	Numeration	Identification	Size	Shape	Type	Abundance	
China	Oyster (*Saccostrea cucullate*)	10% KOH and saturated NaCl flotation	Stereomicroscopy	μ-FTIR	20–5000 μm	Fragments, fibers, sheets, and pellets	[a]PET (34%), PP (19%), PE (14%), PS (8%), CP (8%), PVC (6%), PA (4%), EPS (3%), and others	1.5–7.2 items/g (wet weight [WW]) or 1.4–7.0 items/individual	(Li et al., 2018a)
China	Oysters (*Crassostrea gigas, C. angulate, C. hongkongensis, and C. sikamea*)	10% KOH and 30% H_2O_2	Stereomicroscopy	μ-FTIR	20.34–4807.22 μm	Particles, fragments, films, and fibers	CP (41.3%), PE (22.97%), PET (15.19%), PP (9.89%), PA (4.95%), PS (2.47%), PC (1.77%), and PVC (1.41%)	0.62 items/g WW and 2.93 items/individual	(Teng et al., 2019)
China	Oysters (*C. angulate and Saccostrea*)	30% H_2O_2 and NaCl flotation	Microscopy	μ-RMS	9–1921 μm	Fibers, flakes, fragments, and spheres	PET (69.54%), PP (8.62%), PVC (5.57%), HDPE (4.60%), PS (4.02%), PA (2.87%), and PE (2.30%)	3.24 ± 1.02 items/g WW	(Liao et al., 2021)
India	Oyster (*Magallana bilineata*)	10% KOH and saturated NaI flotation	Stereomicroscopy	ATR-FITR and SEM-EDS	0.005–3 mm	Fragments, fibers, and films	PE (92%), PP (4%), and unidentified particles (4%)	0.81 ± 0.45 items/g WW and 6.9 ± 3.84 items/individual	(Patterson et al., 2019)
USA	Oyster (*C. virginica*)	10% KOH and 4.4 M NaI flotation	Visual inspection and Stereomicroscopy	[b]–	Average 1.64 mm (±1.28) for fibers and 0.405 (±0.521) for fragments	Fragments, fibers, and beads	–	0.18 items/g WW and 0.72 items/individual	(Keisling et al., 2020)
China	Oyster (*S. cucullata*)	10% KOH and 30% H_2O_2	Optical microscopy	μ-ATR-FTIR	1–5000 μm	Fibers, fragments, films, and granules	PET (8.6%), PE (22.9%), PVC (5.7%), PP (11.4%), PS (11.4%), nylon (2.9%), rayon (20%), and others (5.7%)	0.14–7.90 items/g WW	(Wang et al., 2021)
Vietnam	Oyster (*C. gigas*)	10% KOH, 30% H_2O_2, and NaI flotation	Visual verification	μ-FTIR	22.4–1318.8 μm	Fragments, fibers, and beads	Nylon (50.56%), PVA (2.70%), PET (1.12%), and others	1.88 ± 1.58 particles/g WW and 18.54 ± 10.08 particles/individual	(Do et al., 2022)
Argentina	Oyster (*C. gigas*)	22.5 M HNO_3; 30% H_2O_2	Microscopy	–	–	Fibers, fragments, pellets, and beads	–	2–7 particles/individual	(Severini et al., 2019)
South Korea	Oyster (*C. gigas*)	Lipase, protease, and NaCl flotation	–	μ-ATR-FTIR	50–1000 μm	Fragments and fibers	PE, PP, PS, nylon, polyester, acrylic, PVA, PU, alkyd, POM, PVC, and SB	1.13 ± 0/84 particles/g WW	(Jang et al., 2020)
Tunisia	Oyster (*C. gigas*)	10% KOH and NaCl flotation	Stereomicroscopy	ATR-FTIR	0.05–5 mm	Fibers, fragments, and films	PE (30%) and PP (70%)	1.48 particles/g WW	(Abidli et al., 2019)
USA	Oyster (*C. gigas*)	30% H_2O_2 and 25% saline solution flotation	Visual sorting and stereomicroscopy	RMS, and ATR-FTIR	50–150 μm	Spheroids, fibers, and shards	PS, PP, and PE	1.75 particles/individual	(Martinelli et al., 2020)
China	Oyster (*C. gigas*)	30% H_2O_2 and saturated NaCl flotation; 67% HNO_3 and 30% H_2O_2	Stereomicroscopy	μ-FTIR and SEM-EDS	–	Fibers and fragments	Polyester, AHR, PE, PET, and CP	4.53 items/g WW and 24.49 items/g (dry weight [DW])	(Zhu et al., 2020)

(*continued on next page*)

Table 1 (*continued*)

Location	Species	Analysis			Microplastics				Reference
		Digestion	Numeration	Identification	Size	Shape	Type	Abundance	
Australia	Oyster (*S. glomerata*)	10% KOH	Stereomicroscopy	I: μ-FTIR, II: Py-GC–MS	I: 159–2236 μm; II: < 1 μm, 1–22 μm, and > 22 μm	I: Fibers	I: PP, polyacrylamide, nylon, and CE II: PS, PE, PVC, and PP	I: 0.2 items/g WW and 1.2 items/g DW	(Ribeiro et al., 2021)
India	Oyster (*M. bilineata*)	Proteinase K	Stereomicroscopy	RMS	< 4 mm	Fibers, fragments, films, foams, pellets, and rounds	PP and HDPE	–	(Joshy et al., 2022)
USA	Oyster (*C. virginica*)	3% Alcalase; 69% HNO_3; 5% HCl	–	RMS	110–2300 μm for fibers, 14–1280 μm for fragments, and 6–282 μm for beads	Fragments, fibers, and beads	PP, PET, and PS	5.6–7 items/g WW and 104–140 items/g DW	(Aung et al., 2022)

[a] Abbreviations: PET, polyethylene terephthalate; PP, polypropylene; PE, polyethylene; PS, polystyrene; CP, cellophane; PVC, polyvinyl chloride; PA, polyamide; EPS, expanded polystyrene; HDPE, high-density polyethylene; PVA, polyvinyl acetate; PU, polyurethane; POM, polyoxymethylene; SB, styrene butadiene; AHR, aromatic hydrocarbon resin; CE, cellulose; PMMA, poly (methyl methacrylate); TEM, transmission electron microscopy.
[b] "–", not reported in the article.

these reagents can damage or destroy the pH-sensitive polymers. Strong acids can dissolve polystyrene (PS), polypropylene (PP), polyethylene terephthalate (PET), low-density polyethylene (LDPE), and high-density polyethylene (HDPE), resulting in the destruction of polyamide (PA) and color changes of most polymers (Thiele et al., 2019). Aung et al. (2022) confirmed that HNO_3 can completely fuse blue polyethylene (PE) MPs because of its strong acidity. HCl is inefficient in digesting biogenic compounds and can distort the surfaces of polyethylene terephthalate and polyvinyl chloride (Tirkey & Upadhyay, 2021). Strong acids also have a dramatic effect on nylon fibers and cause total loss of this type of polymer during extraction (Li et al., 2015). Therefore, this method is not recommended.

3.1.2. Alkaline digestion

Alkaline digestion (KOH and NaOH) is the most commonly used and recommended method for the rapid degradation of biological tissues. Alkaline solutions can effectively dissolve soft tissues by hydrolyzing fats and proteins, with no obvious impact on polymers (Thiele et al., 2019). Gulizia et al. (2022) revealed that treatment with alkaline reagents, regardless of treatment time and temperature, did not change the physicochemical properties of the tested MPs. Nevertheless, some studies have reported that alkaline treatment causes physical damage and MP discoloration. Cole et al. (2014) demonstrated that alkaline hydrolysis results in structural damage to nylon fibers, fusion of PE fragments, and discoloration of unplasticized polyvinyl chloride granules. However, compared to acid digestion, alkaline digestion is still a recommended approach after considering the trade-off between digestion efficiency and impact on MPs.

3.1.3. Oxidative digestion

Hydrogen peroxide (H_2O_2) has also been used to digest organic materials prior to the isolation of MPs because of its ability to remove other organic materials that interfere with the quantification of MPs. It has limitations, however, including incomplete soft tissue digestion and excessive foaming, which blocks the filter holes, causing underestimation of MPs extracted from samples. Severini et al. (2019) affirmed that treatment of oyster gut samples with H_2O_2 caused incomplete digestion, generating tissue and lipid residues that hindered the quantification of the MPs and suggested a lower abundance. These results are similar to those reported by Thiele et al. (2019) who demonstrated that H_2O_2 is not appropriate for oyster digestion owing to excessive foaming and, therefore, possible sample loss.

3.1.4. Enzymatic digestion

Enzymatic digestion is generally an appropriate alternative for chemical treatment because of its negligible effect on polymers and better extraction efficacy. Proteinase-K has been commonly used to digest organic tissues, with high digestion rates of up to 97.7% in plankton-rich seawater samples, and no damage to MPs (Cole et al., 2014). However, several major issues should be considered when applying enzymes: 1) some industrial enzymes are very costly, and application of enzyme digestion methods may result in unacceptable costs; and 2) enzyme digestion is usually performed in numerous steps, which may increase the possibility of airborne contamination affecting the discrimination of polymers in oysters. In addition, the effectiveness of some enzymatic digestion methods has been estimated solely based on smaller-sized organic tissue samples; for instance, in the study of Thiele et al. (2019), only 0.2 g of sample was digested with Proteinase-K. The cost of digesting entire oysters would likely to pose a substantial economic barrier. Moreover, digestion of only a few samples may result in an underestimation of the abundance of MPs in the samples.

Recently, new attempts have been made to optimize enzyme digestion. Porcine pancreatic enzymes, a cocktail of lipase, amylase, and protease, have been used for bivalve digestion and shown to provide an efficient and gentle digestion method for removing organic tissues (von Friesen et al., 2019). Industrial proteases can also be used to digest organisms; and exhibit high digestion efficiency, polymer recovery, and the ability to maintain the integrity of MPs. However, not all enzymes are suitable for the digestion of oysters. The proteinase Alcalase was used to digest oysters because protein accounts for a high proportion of the tissue make-up in oysters; however, Alcalase did not digest the oyster tissue completely (Aung et al., 2022). Although enzymatic digestion is advantageous for the extraction of MPs from biological samples, challenges in selecting a cost-effective enzyme still need to be addressed. Overly cumbersome steps associated with enzyme digestion can also lead to the loss and underestimation of MPs, and thus should be avoided.

3.2. Quantification of MPs

3.2.1. Dissection microscopy (or stereomicroscopy)

To enumerate MPs in samples, the samples are generally filtered using filtration membranes post-digestion to first enrich for MPs. Once the filters were dry, the numeration and categorization of MPs gathered on the filters could be performed. The first examination of larger MPs is generally performed by visual observation, which can be achieved by naked-eye observation. Criteria that have been proposed to improve the accuracy of quantification during visual enumeration include the following: (1) MPs should not show any associated cellular or organic structures, (2) fibers should be equally thick throughout the entire length, (3) MP color should be clear and homogeneous, and (4) if MPs are transparent, they should be observed under an optical microscope at high magnification to exclude an organic origin (Hidalgo-Ruz et al., 2012; Qiu et al., 2016).

For small MPs, a dissection microscope was used to visually count the number of MPs collected from the filtering membranes. This fast, undemanding, and inexpensive method has been used in many studies to count and categorize MPs. However, this type of analysis is generally subjective, inconclusive, and can be misleading for the following reasons: 1) it does not provide information on polymer identity, that is, it cannot discern natural minerals or other biopolymers from MPs and thus leads to misjudgment; and 2) it does not allow for the recognition of fine MPs because of the limited magnification. For instance, Eriksen et al. (2013) visually counted approximately 20% of hypothetical MPs, which were subsequently identified as aluminum silicate using scanning electron microscopy (SEM). Moreover, post-digestion filtering procedures will affect the accuracy of MP quantification. Small plastic fragments attach to the pores of filters, and some pellets cluster together in the networks during the process of filtration (Cai et al., 2020), thus causing MPs to be lost.

Nonetheless, visual quantification associated with dissection microscopy remains a viable approach, especially when other expensive analytical instruments cannot be used. This approach is also important in studies on the effects of specific types of MPs (e.g., size, color, and shape) on marine species, as selected MPs are readily identifiable and distinguishable from other non-plastic polymers.

3.2.2. Fluorescence microscopy

Fluorescence staining is a reliable, convenient, and effective method for quantifying MPs in samples. The principle of fluorescence staining is based on a dye (e.g., lipophilic Nile red) serving as the fluorophore and physically adsorbing onto the surface of lipophilic components in plastics, owing to the hydrophobicity caused by multiple C—H bonds in their polymer chains (Kang et al., 2020; Scircle & Cizdziel, 2020). Different kinds of fluorescent dyes, such as Nile red, safranin T, and fluorescein isothiocyanate (FITC), are used to fluorescently stain MPs to facilitate visual observation. Nile red appears to be the most effective and promising staining method for MPs because it provides a high average recovery of MPs. Nile red staining of MPs has also been used to quantify the ingestion and egestion of MPs in oysters (Choi et al., 2022), and to study the ingestion of microfibers by oyster larvae, owing to the fact that Nile red-stained MPs are visible and distinguishable (Knauss et al., 2022).

Nonetheless, it should be mentioned that staining efficacy is influenced by factors such as dye concentration and temperature. Higher or lower dye concentrations can interfere with the fluorescence signal. Lv et al. (2019) showed that the fluorescence intensity of Nile red and FITC increased and then decreased with increasing dye concentration because the dye molecules aggregated owing to intermolecular interactions, thereby resulting in a decrease in fluorescence intensity or even quenching at higher concentrations. Time and temperature also influence fluorescence staining efficacy. Typically, the staining incubation time ranges from 5 min to >24 h at temperatures ranging from 30 °C to 60 °C, conditions that promote absorption of the dye by the polymers (Shruti et al., 2022). Moreover, some restrictions remain when applying this method: 1) dye molecules are readily desorbed from MPs as they are physically absorbed on the surface of the polymers; 2) the staining method requires MPs to be dispersed in the staining solution; however, some types of MPs, such as PS, are prone to float on the surface of the solution owing to their low density; 3) staining with single dyes usually does not lead to staining of all types of MPs because they show high (or low) hydrophilicity or hydrophobicity; thus, many MPs are ignored in the quantification process; and 4) co-staining of residual organic tissues caused by incomplete digestion occurs along with that of MPs, causing deviation in the counting of MPs in the samples.

New methods have been developed to overcome these limitations of fluorescence staining. Lv et al. (2019) proposed an effective and rapid staining approach based on the thermal expansion and contraction characteristics of the MPs. This method yields an extended fluorescence intensity time and there are no limitations in the selection of dyes, as well as no special requirements regarding the chemical properties of common MPs. Similarly, Prata et al. (2021) also proposed a new treatment method to effectively remove natural organic matter from biological samples for better Nile red staining and quantification efficiency. Although not all limitations have been resolved, fluorescence staining remains an effective and reliable method of choice compared to dissection microscopy for detecting MPs.

3.3. Identification of MPs

The identification of MPs is routinely the succeeding step after extraction and numeration (Fig. S1). This is necessary because visual quantification using dissection microscopy easily results in false-identification of MPs, and combined staining of residual biological tissues caused by incomplete digestion often results in the overestimation of MPs. Therefore, the results can be biased, leading to inaccurate estimates of the real pollution level of MPs in oysters and aquatic environments. To obtain more accurate information concerning the abundance of MPs in oysters, various techniques have been employed to confirm the MPs, including Fourier transform infrared spectroscopy (FTIR), Raman microspectroscopy (RMS), and SEM. Therefore, in the following sections, we summarize and compare widely used techniques for the identification of MPs in oysters (Table S1).

3.3.1. FTIR

One of the most widely used methods for the detection of MPs is FTIR owing to its high reliability and non-destructive procedure. FTIR provides unique infrared information regarding the specific chemical bonds of polymers. As different materials have different chemical bond compositions, unique spectra are produced that can be used to discriminate plastics from other organic and inorganic substances by comparing their spectra with those of known materials in a well-established polymer spectrum library. FTIR not only enables the accurate verification of MPs, but also the identification of specific types of MPs. For example, using FTIR analysis, 129 of 157 selected polymers isolated from oysters were demonstrated to be real MPs using a dissecting microscope, with the main type being nylon (Jahan et al., 2019). Additionally, as a non-destructive identification method, FTIR offers information concerning the physiochemical weathering degree of MPs through evaluating their oxidation intensity (Tirkey & Upadhyay, 2021). Nevertheless, FTIR is limited in that it can only confirm MPs with sizes > 20 μm. Moreover, verification of the identity of suspected MPs using FTIR can be a time-consuming process when samples are in large quantities as they need to be dried prior to analysis.

Micro-FTIR (μ-FTIR) has been increasingly employed to confirm the presence of MPs and overcome the drawbacks of FTIR. μ-FTIR allows the identification of MPs with a size < 10 μm (Tirkey & Upadhyay, 2021). However, μ-FTIR instruments are cost-intensive, and this technique needs to be performed manually or semiautomatically; thus, high operational abilities and experience are required for operators. Attenuated total reflectance FTIR (ATR-FTIR) is another reliable and fast identification tool for detection of MPs, which facilitates the direct analysis of larger (>500 μm) and irregularly shaped particles without an extra sample extraction process (Wang & Wang, 2018). Owing to its characteristics of surface contact analysis, the pressure introduced by the ATR probe may destroy aged or fragile microplastics; in turn, an ATR probe made of germanium may be damaged by hard inorganic particles (Shim et al., 2017). Nevertheless, (μ- or) FTIR remains an effective and promising tool for the identification of MPs.

3.3.2. RMS

Another non-destructive analysis method, RMS, has been used for the detection of MPs. Identification of MPs using RMS involves irradiating a suspicious sample with a monochromatic laser beam, producing variable frequency of backscattered light due to adsorption, reflection, or scatter by the specific molecular structure and atomic composition of the samples (Fytianos et al., 2021). In terms of the detection of MPs, RMS is comparable to FTIR, including the confirmation of genuine MPs from suspicious polymers and identification of polymer composition. For instance, only 8 of 447 examined polymers from oysters were validated to be MPs with the help of RMS (Martinelli et al., 2020). Compared with FTIR, RMS performs better with tiny microplastics (as small as 1 μm) and delivers chemical and structural information (Tirkey & Upadhyay, 2021). In addition, RMS can offer higher spatial resolution, narrower spectral bonds, and a wider spectral range (Wang & Wang, 2018). RMS can be used to analyze wet samples, and the non-contact analysis of RMS provides the advantage that the MP samples remain intact and can be used for further analysis (Shim et al., 2017).

However, the limitations of RMS must also be considered. The primary drawback of RMS is that it is highly sensitive to the presence of color, additives, and pollutants attached to microplastics, which may interfere with the accuracy and reliability of MP identification. Similar to FTIR, RMS is also a time-consuming method and poses economic challenges when a large number of samples must be analyzed. New approaches have been developed to facilitate MP identification using RMS. Compared to RMS, surface enhanced Raman spectroscopy (SERS) has an advantage of stronger Raman signal (Mikac et al., 2023) and can also be used for the identification of MPs. SERS facilitates the detection of MPs < 1 μm in size. In the study of Hu et al. (2022), SERS-based method employing Ag nanoparticles and potassium iodide for detection of PS nanoparticles is developed. PS particles of four sizes (50, 100, 200, and 500 nm) can be sensitively detected using this method. In conclusion, RMS is a reliable and effective approach for MPs. Similarly, a combined method involving membrane filtration and SERS to detect PS nanoparticles is proposed (Yang et al., 2022). In this method, PS particles are enriched by Ag nanowire membrane and particles with sizes ranging from 50 to 1000 nm were successfully identified by Raman mapping.

3.3.3. Other identification methods

In addition to FTIR and RMS, SEM can be employed to identify microplastics. SEM provides a clear and highly-magnified image of a sample by firing a high-intensity beam of electrons at the sample surface, followed by scanning in a raster-scan pattern. High-resolution images of the surface morphology of particles help discriminate MPs from other organic or inorganic particles. Moreover, SEM can be utilized to analyze the degree of weathering of MPs by examining their surface textures, such as fractures and pits (Zhang et al., 2016). The combination of SEM with energy-dispersive X-ray spectroscopy (SEM-EDS) can offer detailed information regarding the elemental composition of MPs, identify and differentiate any inorganic additives they may contain, and recognize tiny particles that are difficult to detect visually (Tirkey & Upadhyay, 2021). Nevertheless, SEM is expensive and not recommended for handling large quantities of samples, which require substantial time and effort for sample preparation and analysis.

The thermos-analytical technique, pyrolysis gas chromatography-mass spectrometry (Py-GC–MS), is another method that has been successfully used in the identification of MPs. This methodology is based on the thermal decomposition of the sample under an inert atmosphere, followed by analysis of the thermal degradation products. The type of polymer can be determined by comparing the obtained sample pyrograms with reference pyrograms derived from known polymer samples. One of the main advantages of Py-GC–MS is that it can directly analyze solid polymers without any sample pretreatment and it is tolerant to variations in shape, size, and contaminants of the particles (Wang & Wang, 2018). However, Py-GC–MS is a destructive technique that prevents subsequent analysis of samples and requires longer measurement time for single analysis of one particle, suggesting limited applicability for bulk sample quantity analysis.

4. Hazardous effects of MPs on oysters

The following part aims at analyzing the adverse effects of MPs on oysters. The analysis and understanding of underlying mechanisms of MPs toxicity are imperative because MPs accumulated in oysters can transfer into the human body through food chain, posing potential threats to human health. However, investigations of MPs toxicity are heavily carried out in laboratory environment and influenced by exposure conditions. Much remains to be known about the effects of MPs on humans through consumption of MPs-contaminated oysters. Therefore, to know the causal hazards of MPs-contaminated oysters on human health, extensive study and assessment of the potential threats of MPs from oysters across the entire diet should be performed (Chaudhry & Sachdeva, 2021).

4.1. Direct hazards

The hazards of MPs on oysters originate from two main sources: (1) direct hazards, that is, MPs themselves and (2) indirect hazards, toxic pollutants adsorbed onto the surface of MPs. Accumulating evidence shows that when oysters are exposed to MPs, a wide range of undesirable consequences can be triggered (Fig. 2), and consumption of MP-contaminated oysters may pose potential threats to human health. However, most knowledge regarding the negative effects of MPs on oysters comes from laboratory experiments carried out under conditions that have predominantly utilized commercialized MPs, such as monodispersed plastic microbeads with concentrations much higher than those found in the real environment. Realistic environmental exposure to MPs might not court negative effects. For example, Revel et al. (2020) found that oysters exposed to environmentally relevant concentrations of MP did not induce biological effects. However, given the increasing concentration of MPs in the marine environment, and the fact that accumulation of MPs by oysters is a long-term process, research concerning the biological effects of MPs on oysters is still imperative. Hence, to better assess the risk to humans of consuming MP-contaminated oysters, we collectively reviewed recently published studies and discussed the negative effects ascribed to MP exposure in the following sections (Table 2).

4.1.1. Oxidative stress

The absence of enzymes and enzymatic pathways to break down MPs leads to the accumulation of MPs in oysters, which induces the overproduction of reactive oxygen species (ROS) and initiates oxidative stress. ROS, including H_2O_2, hypochlorous acid/hypochlorite (HOCl/ClO^-), hydroxyl radicals (–OH^-), superoxide anion radicals (O_2^-), and singlet oxygen (O^{2-}), are chemically reactive oxygen-containing species produced from molecular oxygen (O^{2-}) during vital processes occurring in organisms (Kwon et al., 2021). Excessive ROS production is harmful because it induces oxidative stress, DNA damage, and even cell death. MPs can induce oxidative damage and disrupt metabolic balance by catalyzing the overproduction of ROS. To prevent oxidative damage, oysters counteract stress by releasing various antioxidant enzymes, such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx), which help maintain redox balance. The effects of MP exposure and oxidative stress on glutathione S transferase (GST), SOD, CAT, GPx, and total glutathione (TG) have been studied in oysters. Teng et al. (2021a) and Revel et al. (2020) observed increases in SOD activity in the digestive gland of oysters exposed to MPs, suggesting the production of ROS since SOD plays an important role in the transformation of excessive O_2^- into less toxic H_2O_2. CAT protects cells against oxidative damage by converting H_2O_2 produced by diverse metabolic processes to less toxic water and oxygen molecules (Sendra et al., 2021). CAT activity

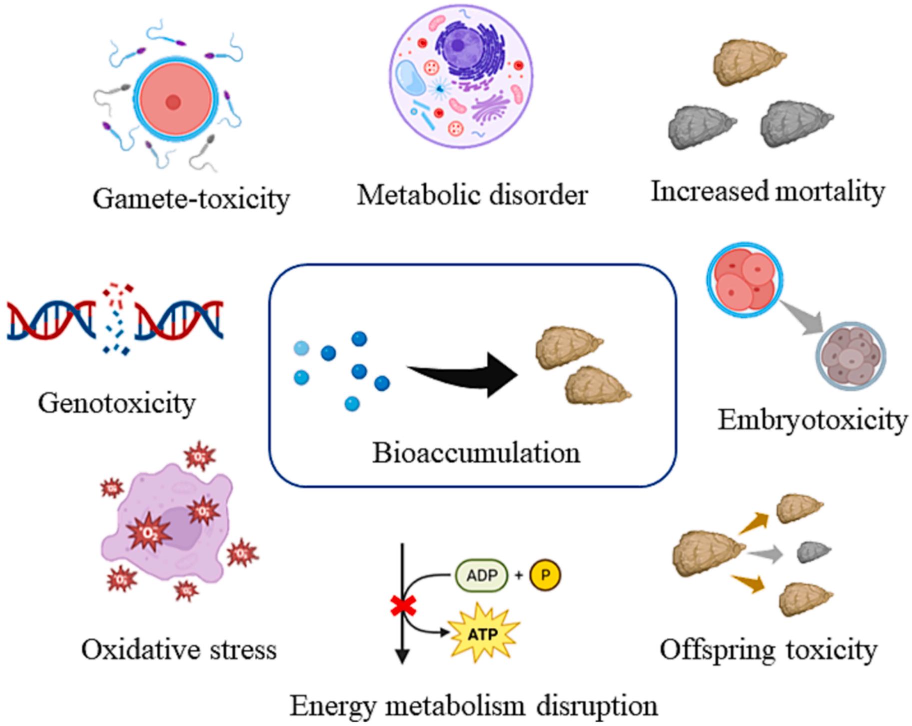

Fig. 2. A range of hazards induced by microplastic bioaccumulation in oysters.

can be either activated or inhibited by MPs, depending on the exposure dose, time, and the effect of MP exposure on the antioxidant system. For example, an increase in CAT activity was initially observed in oysters exposed to MPs (Revel et al., 2020), whereas CAT activity was significantly inhibited in the digestive gland after 21 d of exposure to MPs (Teng et al., 2021a). It could be inferred that short-term exposure to MPs triggers an increase in the activity of several enzymes associated with detoxification, whereas long-term exposure may impair the detoxification and oxidative responses of oysters.

GST is also important for detoxification because it is involved in the biotransformation of exogenous contaminants by generating complexes that are readily discharged. GST protects tissues from oxidative damage through indirect intervention by controlling the level of reduced glutathione to remove excessive ROS (Bringer et al., 2022). Bringer et al. (2022) found that GST activity significantly increased when oysters were exposed to environmentally aged MPs. An increase in GST activity can be used as a defensive mechanism to prevent the internalization of MPs, as well as to prevent oxidative stress that might cause immunotoxicity. GPx protects cells from oxidative damage by catalyzing the reduction of hydroperoxides to water and stable alcohol via oxidation of reduced GSH into its disulfide form (GSSG). GSSG is a strong reducing agent that can help overcome oxidative damage induced by ROS, thereby maintaining the redox balance in the cell (Mkuye et al., 2022). However, a study by Nobre et al. (2020) showed no significant alterations in GPx and GSH when oysters were exposed to MPs, suggesting a weak overall response to MPs.

4.1.2. Energy metabolism disruption

Energy budget refers to the amount of energy available for an individual's maintenance, including general metabolism, tissue growth, and reproduction. An increase in the energy used for an individual's maintenance when external stresses occur, if not compensated by an equivalent increase in energy intake, means that the organism must sacrifice some of the energy allocated to other functions, such as growth or reproduction, to mitigate the increase in energy cost (Gardon et al., 2018). The ingestion of MPs might increase satiety and disrupt the feeding activity of oysters, which would result in decreased energy reserves and disrupt metabolic or energy balance. Gardon et al. (2018) observed that the ingestion rate of microalgae in PS-exposed oysters decreased by 90%. Ingestion rate is an important factor influencing scope for growth, as it is the main process responsible for energy gain in oysters. Moreover, microalgae assimilation by exposed oysters was disrupted by PS exposure. To compensate for the energy deficit caused by the decrease in ingestion rate and assimilation efficiency, the metabolic rate might be reduced. However, the metabolic rate of oysters in this study showed no significant alterations based on respiration rate, suggesting that energy allocated to growth and reproduction was sacrificed to compensate for the individual's maintenance. Similarly, in a study by Gardon et al. (2020) long-term exposure to PS impaired individual fitness and induced energy budget deficits in pearl oysters because no compensatory mechanisms were satisfactory with respect to the total energy budget. The energy budget decline led to lower energy investment in the biomineralization process, ultimately affecting pearl quality and oyster growth. Furthermore, Teng et al. (2021b) observed a significant increase in pyruvate kinase (PK) activity following MPs exposure. An increase in activity of PK, a rate-limiting glycolytic enzyme that catalyzes ATP formation, suggests that oysters can switch to anaerobic metabolic pathways to produce ATP for individual maintenance under stress conditions. This phenomenon could be ascribed to the disruption of feeding activity by the presence of MPs, resulting in a decrease in energy reserves in oysters. In addition, it should be noted that the disruption of energy balance would have negative effects on several metabolic pathways, such as lipid metabolism (Nobre et al., 2020), the tricarboxylic acid cycle, and glucose metabolism (Teng et al., 2021b).

4.1.3. Genotoxicity

In addition to immunotoxicity and energy balance disruption, genotoxic effects of MP exposure have also been reported. MPs can modulate the expression of genes, including upregulation and downregulation. Modulation of gene expression after exposure to MPs might impact the survival and growth of oysters. Gardon et al. (2020) reported that some energy stress- and immune-related genes, such as *gst*, a marker of oxidative stress, were significantly downregulated in oysters

Table 2
Direct hazards of microplastics on oysters.

Species	Microplastics				Exposure period	Consequences	Reference
	Type	Size	Shape	Concentration			
Oyster (*Crassostrea gigas*)	[a]PE and PET	[b]–	Irregular shape	10 and 1000 μg/L	21 days	Inducing oxidative stress; altering lipid and glucose metabolism processes	(Teng et al., 2021a)
Oyster (*C. gigas*)	PE and PP	<400 μm	–	0.008, 10, and 100 μg particles/L	10 days	No significant effects	(Revel et al., 2020)
Oyster (*C. gigas*)	PE and PET	PE: 10.7–93.9 μm; PET: 9.0–56.0 μm	Irregular shape	10 and 1000 μg/L	21 days	Inhibiting lipid metabolism; activating energy metabolism enzyme activities; promoting histopathological alterations; disturbing the integrity of oyster cell membranes	(Teng et al., 2021b)
Oyster D-larvae (*C. gigas*)	PE	4–6, 11–13 and 20–25 μm	–	0.1, 1, and 10 mg particles/L	24 h	Malformations and developmental arrest; impairing maximum swimming speed of oyster D-larvae; inducing abnormal swimming behavior	(Bringer et al., 2020b)
Oyster D-larvae (*C. gigas*)	Fluorescent MPs	1–5 μm	Microspheres	0.1, 1, and 10 mg particles/L	24 h	Inducing developmental abnormalities; alteration in swimming speed and trajectories; causing developmental arrest post-24 h exposure	(Bringer et al., 2020a)
Oyster (*C. gigas*)	PS	2 and 6 μm	Microspheres	0.023 mg/L	2 months	Causing feeding modifications and reproductive disruption; impairing larval development	(Sussarellu et al., 2016)
Pearl Oyster (*Pinctada margaritifera*)	PS	6 and 10 μm	Microbeads	0.25, 2.5, and 25 μg/L	2 months	Impacting assimilation efficiency and energy balance; exerting negative repercussions on reproduction	(Gardon et al., 2018)
Pearl oyster (*P. fucata*)	PE	20 μm	–	0.2, 0.5, and 1.0 g/L	96 h	Hindering growth of $CaCO_3$ crystals; impacting appearance of biominerals and expression of biomineralization-related genes	(Han et al., 2022)
Oyster (*C. gigas*)	PE, PP and PVC	138.6 ± 2.3 μm	–	0.1 and 10 mg/L	2 months	Increasing mortality rate, oxidative stress, and lipid peroxidation	(Bringer et al., 2022)
Pearl oyster (*P. Margaritifera*)	PS	6 and 10 μm	Microbeads	0.25, 2.5, and 25 μg/L	2 months	Inactivation of detoxification and oxidative responses; reduction in total energetic budget; up-regulation of stress-related genes	(Gardon et al., 2020)
Eastern Oyster (*C. virginica*)	PS	3 μm and 50 nm	Microbeads	50 μg/L	48 h	Potential bioreactivity and sublethal impacts	(Gaspar et al., 2018)
Oyster (*O. edulis*)	PLA and HDPE	PLA: 65.6 μm; HDPE: 102.6 μm	Microbeads	2.5 and 25 μg/L	50 days	Filtration rates increased	(Green et al., 2017)
Oyster (*C. gigas*)	PS	6 μm	Microbeads	10^4, 10^5, and 10^6 particles/L	80 days	Condition Index increased initially but reduced over time; lysosomal stability reduced; increased mortality	(Thomas et al., 2020)
Oyster (*C. gigas*)	HDPE	20–25 μm	Microparticles	112 particles/mL	24 days	Valve micro-closures increased; Valve Opening Duration (VOD) and shell growth decreased	(Bringer et al., 2021b)
Oyster (*C. gigas*)	PS	50 nm, 500 nm, and 2 μm	Microbeads	0.1, 1, 10 and 25 μg/mL	1.5, 24, and 36 h	Nanoplastics (NPs) induced a decrease in fertilization success and caused developmental arrest; no significant effects in MPs	(Tallec et al., 2018)
Oyster (*C. gigas*)	HDPE, PP and PVC	138.6 ± 2.3 μm	–	0.1 and 10 mg particles/L	7 days	Lower binding rate to substrate occurred, inducing growth retardation; increased energy consumption	(Bringer et al., 2021a)
Oyster (*C. gigas*)	PS	70 nm–20 μm	Microbeads	1000 particles/mL	8 days	No significant effects on growth of oyster larvae	(Cole & Galloway, 2015)
Oyster (*C. gigas*)	Fluorescent PS-COOH and PS-NH_2	100 nm	Microbeads	0.1, 1, 10, and 100 mg/L	1, 3, and 5 h	Microparticles adhesion; relative cell size and complexity increased	(González-Fernández et al., 2018)
Oyster (*C. gigas*)	PS-NH_2	50 nm	Microbeads	0.1 μg/mL	24 h	Increase in cardiolipin content; slight growth reduction on larvae; no significant effects on growth, reproduction, and ecophysiological performances in adult oyster	(Tallec et al., 2021)

[a] Abbreviations: PET, polyethylene terephthalate; PP, polypropylene; PE, polyethylene; PS, polystyrene; CP, cellophane; PVC, polyvinyl chloride; PA, polyamide; EPS, expanded polystyrene; HDPE, high-density polyethylene; PVA, polyvinyl acetate; PU, polyurethane; POM, polyoxymethylene; SB, styrene butadiene; AHR, aromatic hydrocarbon resin; CE, cellulose; PMMA, poly (methyl methacrylate).
[b] "–", not reported in the article.

after microPS exposure. Downregulation of the expression of these immune-related genes may lead to impaired detoxification and antioxidative processes. Teng et al. (2021a) observed that the expression of genes related to lipid and aerobic metabolism and apoptosis were upregulated in oysters after exposure to MPs. Similarly, variations in the mRNA levels of lipid-related proteins, such as enzymes associated with fatty acid oxidation, have been observed in oysters after MP exposure (Sussarellu et al., 2016). Therefore, alterations in gene expression may affect lipid and glucose metabolism in oysters. Exposure to MPs can influence shell and pearl formation by interfering with the expression of shell biogenesis-related genes. For instance, Han et al. (2022) found an increase in the expression level of multiple biomineralization-related genes, such as nacreous layer-related genes (*N16, Pif, PfN23, and MSI60*), after MP exposure. The high expression of biomineralization-related genes could influence the biomineralization process and growth of the prismatic and nacreous layers of oysters.

4.1.4. Reproductive and developmental toxicity

To better understand the real impacts of MPs on oysters, it is important to estimate the effects of MPs on oysters during the entire life cycle of gametogenesis, fertilization, embryogenesis, larval development, and metamorphosis to the adulthood (Sendra et al., 2021). Short-term exposure to MPs can trigger deleterious effects on the early life stages of oyster. However, most studies on the effects and impacts of MPs have focused on adult organisms, and much remains to be learned about the effects of MPs on oyster reproduction.

MPs can exert profound toxicity on the reproduction and growth of oysters, in which gametes, embryos, and offspring are the main study objects. Deleterious effects on the gametes of oysters (including spermatozoa and oocytes) by MP exposure have been studied. For instance, Sussarellu et al. (2016) observed a significant reduction in oocyte number (−38%), diameter (−5%), and sperm velocity (−23%) of oysters after exposure to micro-PS for 2 months. Bringer et al. (2022) obtained similar results, showing that the quality of oocytes was poorer in mature oysters after exposure to MPs than in the controls. Moreover, González-Fernández et al. (2018) also showed that microparticles caused an increase in relative cell size and complexity owing to the adhesion of particles to oyster spermatozoa. The effects of MPs on the fertilization success between gametes were also estimated. Tallec et al. (2018) found that both 50 and 500 nm microparticles induced a decrease in fertilization rate compared to controls. Similarly, Sussarellu et al. (2016) demonstrated a 23% reduction in sperm velocity in oysters exposed to MPs, which may lower their fertilization ability. In addition, sex determination in oysters can also be impacted by MPs and the size and age of the oyster. For example, it was found that most MPs-contaminated oysters were transformed into males rather than females, unlike the controls, when they reached the sexual maturation stage (Bringer et al., 2022). Sorini et al. (2021) also observed a significant shift in the sex ratio after long-term exposure to PET particles. This may be because MPs disrupt sex-specific reproduction and decrease sex hormone secretion in females via inhibition of synthesis (Wang et al., 2019).

In addition to direct damage to the gametes of oysters, MPs also impair embryo development. Embryogenesis is a momentous initial period in the life cycle, and adverse external stresses can lead to hypoxia, deformity, and dysfunction. Owing to the characteristics of external fertilization, early life stages, such as gametes and embryos, must deal with external adverse factors. MPs that occur in the environments where oysters live may pose threats to the survival of embryos. Tallec et al. (2018) found that PS coated with $-NH_2$ groups induced strong toxicity on both gametes and embryos, and a high concentration of PS coated with –COOH groups caused the death of oyster embryos. A similar sensitivity to MPs was observed in mussel embryos after exposure to PS coated with $-NH_2$ for 48 h (Balbi et al., 2017). It has been speculated that MPs disrupt membrane integrity during cell division, thereby causing retardation during embryonic development.

Exposure to MPs also leads to severe malformations and developmental arrest of oyster larvae. Sussarellu et al. (2016) showed that the D-larvae yield and larval growth of offspring from contaminated parents decreased by 41% and 18%, respectively, after treatment with MPs for two months. Similarly, oyster larvae exposed to two concentrations of MPs (0.1 and 10 mg particles/L) consisting of HDPE, PP, and polyvinyl chloride (PVC) also showed growth retardation (Bringer et al., 2021a). Further, Bringer et al. (2020b) found that the D-larvae length reached 60.3 ± 0.7 μm after treatment with 0.1 mg particles/L, while the average length of larvae at 10 mg particles/L was 55.9 ± 6.3 μm. This phenomenon may be attributed to the MPs forming agglomerations on the mantle and the locomotor eyelashes of larvae, influencing the growth of larvae. Moreover, it seems that the smaller the MPs, the more noticeable the toxic effects on the growth of oyster larvae. Bringer et al. (2020a) reported that smaller PE particles (4–6 and 11–13 μm) induce stronger malformations and developmental arrest than larger particles (20–25 μm). Similarly, Tallec et al. (2018) also demonstrated that 50 nm microparticles induced stronger fertilization and embryogenesis inhibition than particles with sizes of 2 μm. Although several studies have stressed the negative effects of MPs on gametogenesis, fertilization, and embryo-larval growth, divided results have often been reported across experiments. For example, Cole & Galloway (2015) demonstrated that PS particles had limited effects on the size of oyster larvae after 8 d of exposure. It could be speculated that the exposure concentration of MPs was lower in which particles were easily ingested and passed by oyster larvae owing to the limited selectivity and relatively simple feeding organs; thus, it was insufficient to influence the growth of oyster larvae. Additionally, MP exposure influences the settlement success of oyster larvae. For example, oyster larvae treated with MPs at a concentration of 10 mg particles/L exhibited a lower substrate binding rate than that of controls without MPs, which may affect the oyster's ability to grow (Bringer et al., 2021b). MP exposure may also result in a significant increase in mortality rate. Sussarellu et al. (2016) found that exposure of oysters to PS particles induced energy flow disruption, metabolic dysfunction, and increased mortality rate. However, it remains difficult to draw conclusions that the mortality of oysters is completely derived from the lethal effects of MPs rather than from other environmental stresses, such as pathogenic microbes, chemical leachate, and malnutrition. For instance, Gardon et al. (2020) found that leachates from plastic gear led to developmental retardation and increased mortality of embryos of the pearl oyster.

4.2. Indirect hazards

Currently, research on the impacts of MPs is not constrained to evaluating their accumulation in marine species and direct hazards on living organisms, but it is also moving on to investigations of the role of MPs as potential vehicles for a wide variety of toxic substances. MPs can serve as ideal carriers of pollutants in the aquatic environment because of their small dimensions, large specific areas, and strong hydrophobicity (Fig. 3). Through hitchhiking, MPs can facilitate the internalization and bioaccumulation of pollutants in aquatic organisms and aggravate their toxicity. However, research on the impact of co-exposure to MPs and toxic chemicals has been relegated to the periphery of this study area. To better estimate the indirect hazards derived from other contaminants absorbed onto MPs, in the following section we discuss the adsorption of MPs to various pollutants such as persistent organic pollutants, heavy metals, antibiotics, and pathogenic microbes.

4.2.1. Adsorption of persistent organic pollutants (POPs)

In addition to MPs, a wide range of POPs, such as polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), hydrocarbons (HCs), and endocrine disrupting chemicals (EDCs), have been detected in marine environments. POPs are synthetic chemicals that are resistant to natural degradation. POPs distributed in aquatic environments can be ingested and bioaccumulated by marine species. Oros et al. (2005) found that PBDEs could be ingested by bivalves at concentrations of 9–6, 13–47, and 85–106 ng/g in oysters, mussels, and clams, respectively, and higher concentrations of organochlorine compounds and PBDEs were also observed in oysters (Mukai et al., 2020). Moreover, accumulating evidence suggests that long-term exposure to these contaminants at even relatively low concentrations poses a range of hazards, such as neurotoxicity, immunotoxicity, genotoxicity, endocrine disruption, and reproductive disorders (Islam et al., 2018; Tang et al., 2020), and consumed contaminated seafood may pose a huge threat to human health.

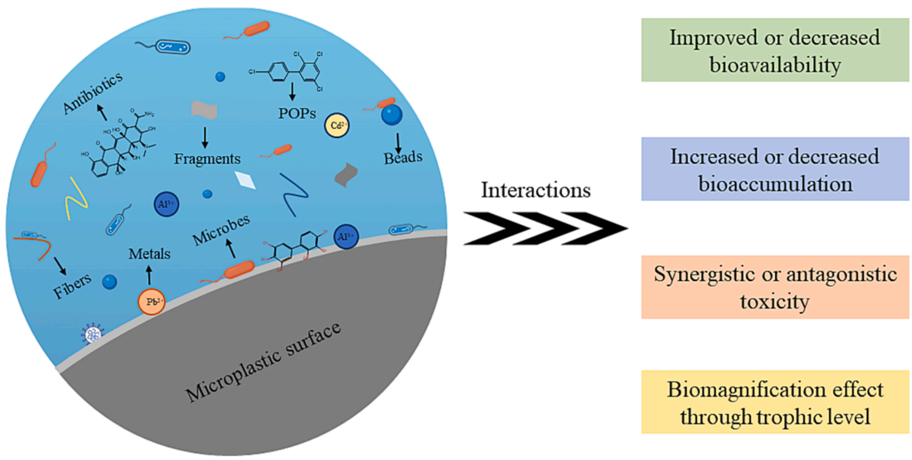

Fig. 3. Adsorption of various contaminants on microplastics and the interactions between them.

Owing to the hydrophobic properties and relatively large surface area of MPs, POPs, preferentially with hydrophobic compounds, tend to adsorb onto plastics, causing a global environmental issue. The ingestion of these MPs by aquatic animals may result in increased bioaccumulation and bioavailability of POPs and potential hazards. Several studies have demonstrated that MPs can serve as vehicles for POPs distributed in the environment. For instance, PCBs, PBDEs, PBDDs, and PCDDs have been found adsorbed onto MP pellets sampled from beaches (Wang et al., 2021). DDTs, hexachlorocyclohexanes (HCHs), and PCBs have been detected in resin particles collected from coasts and beaches (Le et al., 2016; Yeo et al., 2015). Shi et al. (2020) also found that PAHs and organochlorine pesticides (OCPs) were absorbed on MPs sampled from beaches. Among the various types of POPs, PAHs are the most commonly observed in association with MPs. For example, high concentrations of PAHs and PCBs were observed in MPs collected from both inshore and harbor surface waters (Capriotti et al., 2021), which can be attributed to their strong capacity to bind to MPs. Similar results were reported by Van et al. (2012), who found that PS and rubber particles could carry PAHs and PCBs. Mai et al. (2018) also found that PS and PE particles collected from sea surface water carried PAHs with concentrations ranging from 3400 to 119,000 ng/g, and the adsorption level was considerably higher than any field measurements in the present study.

When MPs are ingested by biota, the adsorbed contaminants may be desorbed and accumulate in organisms, after which they may be transferred to higher trophic levels and eventually reach the human body through the food chain, negatively impacting marine species and human health. However, to date, the combined toxicity of MPs and various persistent organic pollutants in shellfish, especially oysters, remains poorly described. Moreover, synergistic toxicity effects may occur in marine animals owing to strong interactions between MPs and POPs. For instance, Tang et al. (2020) first demonstrated that the presence of MPs affected the adverse impacts of benzo[α]pyrene (B[α]P) and 17β-estradiol (E2) on the immune response of clams, with smaller particles aggravating immunotoxicity. In another study of mussels, Avio et al. (2015) found that MPs improved the bioavailability of pyrene, and simultaneous exposure to MPs and pyrene caused stronger neurotoxicity along with irreversible DNA damage compared to the non-contaminated control group. In this regard, MPs could serve as media to facilitate the bioaccumulation of high concentrations of these pollutants through ingestion, subsequently causing serious physiological damage to aquatic animals and exhibiting biomagnification towards humans through trophic level interaction. However, some research results regarding the interactions between MPs and POPs may contradict the findings mentioned above. Pittura et al. (2018) found that both individual exposure to benzo[α]pyrene (B[α]P) and co-exposure to MPs and B[α]P did not induce obvious ecotoxicological effects at the molecular and cellular levels after 28 d exposure. Similarly, combined exposure to PS particles and bisphenol A did not improve the inhibitory effect on acetylcholinesterase activity, which is important for neuronal and neuromuscular transmission in zebrafish (Chen et al., 2017). The inconsistent results concerning the hazards of MPs and POPs suggest complex interactions between MPs and POPs, which require further study.

4.2.2. Adsorption of heavy metals

Oysters are easily enriched with heavy metals, and the hyperaccumulation for multiple metals, such as Cu, Cr, and Cd has been recorded. Metals can exert toxic effects on oysters, including oxidative stress, DNA damage, lipid metabolism dysfunction, immunotoxicity, and reproductive toxicity (Wang et al., 2018).

MPs can act as vectors of heavy metals and exacerbate their internalization of heavy metals in oysters. Elements, such as Ba, Cu, and Cr, are absorbed onto microfibers that accumulate in oysters (Saldaña-Serrano et al., 2022), and Cr, Ni, Mn, Zn, Cd, and Pb are adsorbed onto the surfaces of microplastics isolated from oysters (Zhu et al., 2020). Zhu et al. (2020) found that the *in vivo* concentrations of Cd, Cr, Pb, and Cu enriched in oysters increased with the *in vivo* abundance of MPs, suggesting that the bioaccumulation of heavy metals can be magnified *in vivo* by MPs.

Because both MPs and heavy metals can exert adverse effects on organisms, experiments have been performed to compare and analyze the individual and combined toxic effects of MPs and heavy metals on select species, especially considering that MPs can bio-magnify the harmful effects of heavy metals and pose potential threats to human health. Luís et al. (2015) showed that co-exposure to MPs and Cr resulted in a 31% decrease in acetylcholinesterase activity, whereas individual metal Cr caused an 18–21% decrease in enzyme activity. Zhou et al. (2020) also found that MPs have the potential to increase the bioaccessibility of heavy metals, and the combined exposure to MPs and Cd had higher adverse effects on earthworms. However, co-exposure to MPs and heavy metals may exhibit antagonistic effects. Wang et al. (2019) found that the existence of MPs reduced the accumulation level of As and the transformation rate of As(V) to As(III), weakening the toxicity in earthworms, which can be attributed to the fact that MPs absorbed As and thus mitigated the toxic effects of As on gut bacteria. Therefore, the interactions between MPs and heavy metals should be further clarified to better understand the adsorption mechanisms between MPs and heavy metals.

Some studies have confirmed that the presence of MPs accelerates the accumulation and bioavailability of heavy metals in organisms, and co-exposure to MPs and heavy metals may show synergistic effects.

However, a recent study of Vieira et al. (2021) found that MPs did not have a significant impact on the accumulation and bioavailability of trace metals, such as Cr, Cd, Cu, Pb, and Zn, suggesting that MPs may not be an important transport carrier for heavy metal contamination in oysters. Further studies should focus on the interactions between MPs and heavy metals, as well as the effect of MPs on bioavailability and enrichment of heavy metals in organisms to better assess the effects of combined exposure to MPs and heavy metals on oysters and human health.

4.2.3. Adsorption of antibiotics

Owing to their effective sterilization capacity, veterinary antibiotics have been widely applied in both human medicine and aquaculture. However, it is reported that 30–90% of antibiotics are discharged into the environment in their original or metabolite forms (Carvalho & Santos, 2016), which renders antibiotic pollution as one of the emergent environmental issues. The adsorption of a variety of antibiotics by different types of MPs has been studied previously (Guo & Wang, 2019). Thus, antibiotics and MPs may exert synergistic effects on aquatic organisms. First, the presence of MPs may aggravate the adverse effects of antibiotics. For example, the co-exposure of mussels to PS particles with three types of veterinary antibiotics (oxytetracycline [OTC], florfenicol [FLO], and sulfamethoxazole) remarkably reduced the phagocytic rate and total hemocyte count compared to individual exposure to antibiotics, which thus impacts the immune responses of mussels (Han et al., 2021). Another study on oysters found that exposure of oysters to MPs and triclosan (MPT) significantly increased the activity of ethoxyresorufin-O-deethylase (EROD) compared to individual exposure to MPs or MPT (Nobre et al., 2020). EROD is a biomarker associated with the biotransformation of hydrophobic POPs, and the results showed that co-exposure of MPs with POPs exerted combined effects on oyster physiology and biochemistry. Similarly, Zhou et al. (2021) found that combined exposure to PS particles and two veterinary antibiotics, OTC and FLO, rendered the immune responses of clams more vulnerable to antibiotics, such as increasing intracellular ROS content, suggesting that the co-presence of MPs would aggravate the toxic effects of antibiotics. Therefore, the presence of MPs may improve the bioaccumulation ability of antibiotics in marine species. For example, PS particles remarkably increased the bioaccumulation of OTC and FLO in clams Zhou et al. (2021) and Paul-Pont et al. (2016) found that PS MPs significantly inhibited the excretion of fluoranthene in mussels. It is speculated that the reasons may be ascribed to the following: 1) MPs can carry antibiotics and therefore increase their concentration in marine species when they are ingested, that is, the Trojan horse effect, and 2) the presence of MPs disrupts the metabolic process of antibiotics, which results in a reduction in metabolism and degradation of antibiotics (Wang et al., 2021). Moreover, MPs may increase the risk of antibiotic resistance in marine species, as MPs could play an important role in antibiotic resistance. However, to date, interactions, such as synergistic effects, between MPs and antibiotics in oysters remain poorly understood, and some studies have found that MPs, such as PS particles, may reduce the bioaccumulation of antibiotics in organisms (Yang et al., 2020) and alleviate the neurotoxicity caused by antibiotics (Zhang et al., 2019). Therefore, more efforts should be made to clarify the interactions, especially the combined effects of MPs and antibiotics, to better explore and address the potential threats posed by co-exposure to MPs and antibiotics.

4.2.4. Adsorption of pathogenic microbes

MPs are regarded as persistent reservoirs and potential vehicles for pathogens as they provide factitious niches for the colonization of various communities of pathogenic organisms. Several types of microbes, such as toxic algae, fungi, and bacteria, can attach to MPs (Frère et al., 2018; Gkoutselis et al., 2021). *Vibrio splendidus* is an oyster pathogen that can be absorbed onto a wide range of MPs such as PP, PVC, PE, and PS (Kirstein et al., 2016; Xu et al., 2019; Zhang et al., 2020). Exposure to MPs contaminated with *Vibrio* spp. may threaten oysters and cause infections and outbreaks after oyster consumption. Toxic dinoflagellates, which cause the mortality of pearl oysters, have also been found on the surface of MPs (Zhang et al., 2020). To ensure the safe consumption of oysters, the interactions between MPs and various pathogens, including the survival and transmission of microbes on MPs, should be further investigated.

5. Depuration of MPs in oysters

Generally, depuration is a commercial practice in which shellfish are kept in a tank and exposed to purified seawater for about 2 d to reduce contaminants in shellfish for food safety (Choi et al., 2022). This process is required in many countries, including the United States and Japan, and countries in Europe. Depuration processes exploit the natural filtering ability of shellfish, and have been demonstrated to reduce the level of MPs in oysters (Table 3), thus reducing the probability of transmission of MPs to consumers through the consumption of contaminated bivalves.

Conventional oyster depuration methods mainly use UV light, chlorine, and ozone, as they can disinfect and purify natural or artificial seawater to favor the depuration of contaminants (Fig. S2). However, it should be mentioned that the depuration efficiency of MPs is affected by some factors such as time and MP size. The depuration length of time could be an important parameter because the depuration period varies among marine species and contaminants. Van Cauwenberghe & Janssen (2014) found a reduction of about 33% in MPs in mussels and 25% in oysters after 72 h depuration under similar processing conditions. Similarly, Birnstiel et al. (2019) found that MPs accumulated in wild and farmed mussels decreased by 46.8% and 29.0% post 93-h depuration, respectively. Furthermore, in the depuration of MPs ingested by oysters, particle levels were reduced by 78.6% for fibers, 80.8% for fragments, and 64.2% for crumb rubber after a 96-h depuration period (Weinstein et al., 2022). Additionally, oyster quality is associated with processing time during depuration. Bio & Nunes (2021) observed a significant percentage decrease in condition index of oysters owing to prolonged periods of depuration. Chen et al. (2022a) also found that protein expression declined after a 72-h depuration period, possibly stemming from the excessively long depuration time, leading to excessive stress and quality decline of the oysters. Moreover, a longer depuration time may pose more economic threats to the industry and potentially increase the cost of shellfish to consumers, whereas a shorter period cannot ensure efficient depuration of MPs in oysters. Van Cauwenberghe & Janssen (2014) observed that MP levels in oysters were reduced by 25% after 3 d of depuration, whereas Covernton et al. (2022) found that MPs in oysters were reduced by 73% after 5 d of depuration, but no further reduction was observed at 10 d. Therefore, the most economical and efficient depuration period should be considered to achieve an affordably higher depuration rate.

The depuration rate also varies according to the size of the MPs; smaller particles generally have a higher bioavailability and require longer depuration times than larger ones. In a study of oysters, Graham et al. (2019) demonstrated that majority of MPs were discharged after 72 h depuration, while a portion of the smaller MPs was still retained in the oysters. In a similar study, Ward & Kach (2009) showed that the egestion of 100-nm and 10-μm PS particles ingested by oysters began after 24 h and 6 h depuration, respectively. These phenomena may be ascribed to the fact that finer MPs can enter the digestive gland of oysters and thus have a longer gut retention time, whereas larger particles that cannot be ingested will be covered in mucus and expelled without passing through the digestive tract. Additionally, the effect of microalgae feeding on depuration efficiency should be considered. Feeding during depuration is costly, and its impact on depuration efficiency is not understood. Bagenda et al. (2019) reported that feeding did not significantly improve the efficacy of oyster depuration against V. *parahaemolyticus* and V. *vulnificus*. On the other hand, when algae are

Table 3
Depuration of microplastics in oysters.

Species	Exposure time	Microplastics			Depuration time	Conclusions	Reference
		Type	Size	Concentration			
Oyster (*Magallana gigas*	24 h	Fluorescent [a]PS microspheres	100 ± 7.42, 250 ± 23.2, and 500 ± 52.34 mm	60 particles/L	7 d	MPs reduced by 84.6 ± 2% post-7 d depuration	(Graham et al., 2019)
Oyster, (*C. gigas*)	[b]–	–	–	–	72 h	MPs reduced by 25% post-3 d depuration	(Van Cauwenberghe & Janssen, 2014)
Oyster (*C. virginica*)	96 h	Purple PE fibers, green nylon fragments, and micronized crumb rubber	38–150 μm	5000 particles/L	96 h	MPs reduced by 78.6% for fibers, 80.8% for fragments, and 64.2% for crumb rubber after 96 h depuration	(Weinstein et al., 2022)
Oyster (*C. virginica*)	2 h	(Non–) fluorescent PS microspheres and black nylon microfibers	Microspheres for 19–1000 μm and fibers for 30 μm in diameter	735 microspheres (Experiment 1, 700 mL, all sizes), 495 microfibers (Experiment 2, 700 mL, all sizes), or 34 spheres and fibers (Experiment 3, 700 mL, both sizes)	48 h	MPs ejected increased from 10 – 30% for the smallest ones to 98% for the largest ones	(Ward et al., 2019)
Oyster (*C. gigas*)	–	–	–	–	1, 3, 5, and 10 d	MPs reduced by 73% post-5 d depuration but no further reduction at 10 d	(Covernton et al., 2022)
Oyster (*C. gigas*)	10 d	PE and PP fragments	< 400 μm	0.008, 10, and 100 μg particles/L	10 d	No MPs observed in oyster tissues or biodeposits after 10 d depuration	(Revel et al., 2020)
Oyster (*C. brasiliana*)	–	–	–	–	24 h	No MPs found in depurated oysters	(Christo, 2021)
Oyster (*C. gigas*)	7 d	PS fragments	<50 μm, 50–100 μm, 100–150 μm, 150–300 μm, 300–500 μm, 500–1000 μm, and > 1000 μm	300n/L	7 d	62.9% of the PS particles egested after 7 d depuration	(Choi et al., 2022)
Oyster (*C. madrasensis*)	–	–	–	–	16 h	72.5% of MPs egested after depuration	(Radampola, 2019)
Oyster (*C. virginica*)	45 min	(Non–) fluorescent PS microbeads	100 nm and 10 μm	1000 beads/L	2, 24, 48, and 72 h	10-μm beads egested after 6 h, 100-nm beads egested after 72 h	(Ward & Kach, 2009)

[a] Abbreviations: polyethylene terephthalate (PET), polypropylene (PP), polyethylene (PE), polystyrene (PS), cellophane (CP), polyvinyl chloride (PVC), polyamide (PA), expanded polystyrene (EPS), high-density polyethylene (HDPE), polyvinyl acetate (PVA), polyurethane (PU), polyoxymethylene (POM), styrene butadiene (SB), aromatic hydrocarbon resin (AHR), cellulose (CE), poly (methyl methacrylate) (PMMA).
[b] "–", not reported in the article.

introduced with MPs at the same time, oysters may selectively feed on algae and reject MPs to some extent, owing to food-sorting mechanisms. Revel et al. (2020) found that no PP or PE particles were observed in oyster tissues, whereas MPs were identified in the biodeposits after exposure to MPs for 10 d. This situation may be attributed to the fact that the MPs could not be ingested by oysters owing to their larger sizes and are therefore discharged in the form of pseudofeces. Collectively, these results suggest that the effect of feeding on the depuration efficacy of MPs during depuration requires further study.

6. Future prospects and challenges

After conducting this study, we formulate the following perspectives and challenges to be considered in future studies.

Topic 1: Standardized criteria for analysis of MPs in oysters.

In this study, several methodologies used for MP analysis, including digestion, quantification, and identification are discussed. Although several digestion methods are used to separate MPs from oysters, some of these reagents can damage or destroy polymers, which is inconvenient for the quantification of MPs. Therefore, standard protocols for the extraction of MPs from oysters should be established in the future.

Topic 2: Simulation of environment in accordance with actual MP exposure conditions.

A primary drawback of laboratory studies of MP ingestion is that they use virgin MPs rather than “environmentally realistic” particles (Craig et al., 2022). The MPs used in these studies were typically of specific sizes, undiversified shapes, and high concentrations, which often have limited environmental relevance. Moreover, most studies conducted under laboratory conditions have focused on shorter exposure times. These laboratory conditions often do not reflect the real exposure environment and may exaggerate the hazards posed by exposure to MPs. Future research needs to focus on simulating real MP exposure conditions to provide robust evidence associated with potential hazards from MP exposure.

Topic 3: Further studies on the interactions between MPs and other pollutants.

MPs and other contaminants, such as heavy metals and POPs, are typically included in different classes of contaminants, but these pollutants can be absorbed onto the surface of MPs. The synergistic (or antagonistic) interaction effects between MPs and these pollutants are poorly understood. Thus, more studies are needed on the effects of combined exposure to MPs and other contaminants on certain organisms to estimate their potential threats to humans.

Topic 4: Future perspectives on MP depuration techniques.

Future studies on depuration of MPs should include: 1) investigations of the distribution of MPs in different tissues; most studies tend to involve digesting the whole organism to estimate MP abundance; however, it is important to study the distribution and concentration of MPs in different parts of the body to aid in understanding the transfer and accumulation of MPs in organisms, 2) co-depuration of MPs and other pollutants; in addition to MPs, various contaminants can also be ingested by oysters. Although the concentration of these contaminants can be reduced through depuration processes, depuration efficacy varies depending on the types of contaminants. Combined depuration of MPs and other pollutants should be considered in future studies for better optimization of depuration techniques.

3) an effort to strike a balance between depuration efficiency and quality of oysters; the ultimate objective of the depuration process of shellfish is to enable food security and human health, which means safe consumption by consumers. It should be highlighted that despite changes in depuration parameters, such as temperature and salinity, which may facilitate excretion of contaminants in oysters, fluctuating survival conditions may have negative effects on the quality of oysters. Thus, studies need to not only explore the conditions for improving depuration efficacy, but also maintain a higher quality and living state of shellfish at the same time.

Topic 5: Risk assessment of MPs from consumption of shellfish.

The ingestion and accumulation of MPs in humans have been demonstrated in previous studies. For example, Ragusa et al. (2022) were the first to find that MP contamination occurs in human breast milk. Bivalves, as popular and important aquatic products, are an inevitable source for the ingestion of MPs by humans. Long-term exposure to MPs will have adverse effects on bivalves and pose potential threats to human health; however, the direct hazards from the ingestion of MPs by humans remain unclear. Further, studies are required to estimate the potential hazards of shellfish consumption in humans.

Topic 6: Improvement in plastic waste management and development of sustainable plastics.

To alleviate and tackle the environmental pollution caused by MPs, low-cost, high-quality, and environmentally friendly plastics produced from renewable sources can be a promising alternative to single-use plastics. Additionally, we call for improving plastic waste management and MP management enactment to ensure food security and human health.

7. Conclusions

This review provided an extensive overview of the current studies on microplastic contamination in oysters. The analytical methods currently used for the quantification and identification of MPs are compared and summarized. The results of this study showed that different analytical methods contributed to variance in the quantification of MPs in oysters. Due to the lack of a specific standard method for MPs analysis, harmonization and standardization of methods concerning sample digestion, MPs quantification, and identification are in need. Substantial studies have demonstrated that long-term exposure to MPs induced various adverse effects, such as oxidative stress, genotoxicity, reproductive and developmental toxicity, and even increased mortality in oysters. However, much remains to be known about the direct effects of MPs on human health and their underlying mechanisms. Therefore, there is an urgent need to assess the potential threats posed by the consumption of MP-contaminated oysters to humans in future studies. The depuration process can be used to facilitate the excretion of MPs in oysters. Depuration of MPs in oysters is imperative as MPs accumulated in oysters can be transferred to higher trophic levels and ultimately to humans through the food chain. It was found longer depuration time contributed to higher depuration efficacy. However, many studies concerning MP depuration processes have focused on depuration time without considering other factors or methods, despite their known effects on the depuration efficiency of other contaminants in oysters. Considering that changes in temperature and salinity help the reduction of oyster pathogens, research on the effects of various factors on MPs depuration should be one of the research subjects that ensure the food safety for consumers. In addition, to alleviate plastic pollution and ensure food security, improved plastic waste management and use of biodegradable plastics are recommended to promote the sustainable development of environmental protection and food security.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.136153.

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